Hoechst Stains

Table 1. Contents and storage information.

Product	Amount	Concentration	Storage	Stability
Hoechst 33258, pentahydrate (bis-benzimide) FluoroPure™ grade (MW 623.96) (H21491)	100 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Hoechst 33258, pentahydrate (bis-benzimide) (MW 623.96) (H1398)	100 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Hoechst 33258, pentahydrate (bis-benzimide) (MW 623.96) (H3569)	10 mL	10 mg/mL (16.0 mM) solution in water	• Store at 2–6°C • Protect from light	When stored as directed, product is stable for 6 months.
Hoechst 33342, trihydrochloride, trihydrate, FluoroPure™ grade (MW 615.99) (H21492)	100 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Hoechst 33342, trihydrochloride, trihydrate, (MW 615.99) (H1399)	100 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Hoechst 33342, trihydrochloride, trihydrate, (MW 615.99) (H3570)	10 mL	10 mg/mL (16.2 mM) solution in water	• Store at 2–6°C • Protect from light	When stored as directed, product is stable for 6 months.
Hoechst 34580, (MW 560.96) (H21486)	5 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
nuclear yellow (Hoechst S769121, trihydrochloride, trihydrate, (MW 651.01) (N21485)	10 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Approximate Fluorescence Excitation and Emission, in nm: Excitation/Emission bound to DNA: Hoechst 33258 352/461 nm (see Figure 2); Hoechst 33342 350/461 nm; Hoechst 34580 392/440 nm (see Figure 3); Nuclear Yellow 355/495 nm.				

Introduction

The blue fluorescent Hoechst dyes (Figure 1) are cell permeable nucleic acid stains that have multiple applications, including sensitive detection (>3 ng) of DNA in the presence of RNA in agarose gels,[1] automated DNA determination,[2] sensitive determination of cell number [3,4] and chromosome sorting.[5] The fluorescence of these dyes is very sensitive to DNA conformation and chromatin state in cells. Consequently, they can detect gradations of nuclear damage. The Hoechst dyes are useful vital stains for the flow cytometric recognition of DNA damage [6,7] and other viability measurements by monitoring the emission spectral shifts of the dyes.[8] These bisbenzimidazole derivatives are supravital minor groove–binding DNA stains with AT selectivity.[9] The dyes

bind to all nucleic acids, but AT-rich dsDNA strands enhance fluorescence ~2-fold greater than GC-rich strands. This property has been used to identify Q-bands in chromosomes (Q-bands: AT-rich chromosome regions that fluoresce brightly when stained with the dye quinacrine).[10]

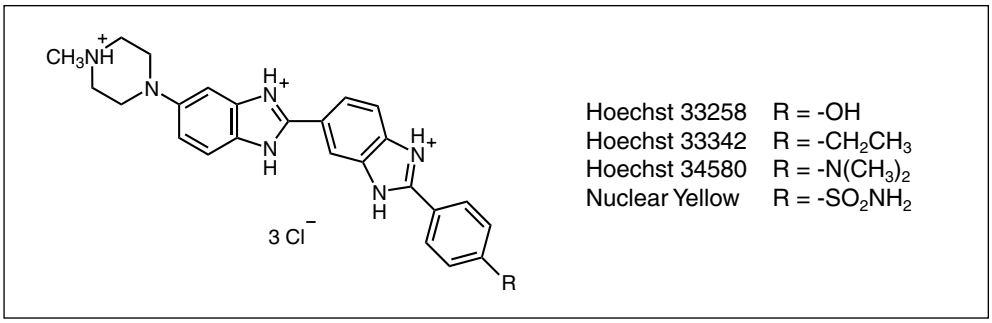

Figure 1. Structure of Hoechst dyes.

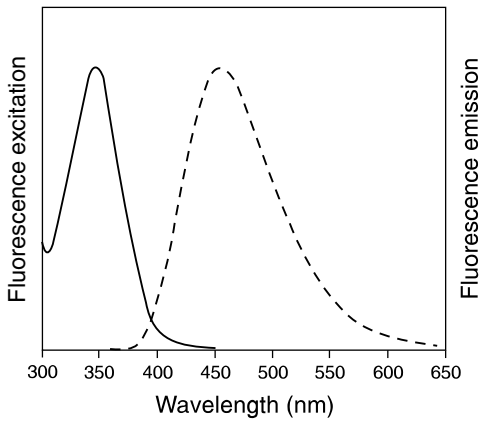

Figure 2. Fluorescence excitation and emission spectra of Hoechst 33258 bound to DNA

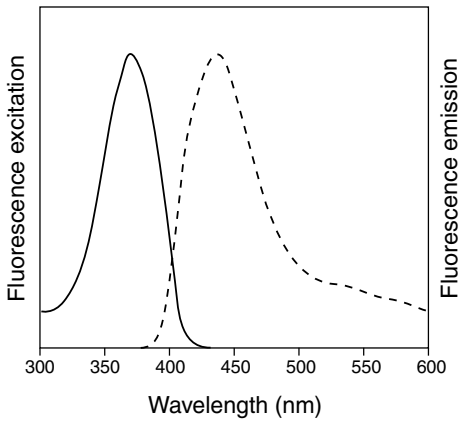

Figure 3. Fluorescence excitation and emission spectra of Hoechst 34580 bound to DNA.

BrdU Quenching

Hoechst 33342 and 33258 are quenched by BrdU.[11,12] Fluorescence is enhanced upon binding to dsDNA at stretches of at least three AT base pairs, but no binding to stretches of two or more GC base pairs. The Hoechst dyes requires a $[dA\text{-}dT]_3$-$[dG\text{-}dC]_1$ sequence to enhance fluorescence, with binding to the bottom of the minor groove as a prerequisite. It is postulated that the bromine on BrdU (or the chlorine on CldU) deforms the minor groove, such that the dye molecule can no longer reach its optimal binding site. It has shown that the Hoechst dyes still bind (with an even higher affinity) to BrdU substituted DNA, but no fluorescence ensues.[13] This has been used extensively to monitor cell cycle progression.

Hoechst 33258 is slightly more water soluble than Hoechst 33342, but both have been used extensively to stain live cells. The products may be used in fluorescence microscopy, microplate, cuvette, and flow cytometry applications. Nuclear yellow (Hoechst S769121) is more commonly used as a neuronal retrograde tracer.[14-16]

Guidelines for Use

Preparing Stock Solutions of Hoechst Dyes

The solid dyes may be dissolved in either water, dimethylformamide (DMF), or DMSO to make concentrated stock solutions up to 10 mg/mL. Stock solutions may be stored refrigerated or frozen, protected from light. Note: The Hoechst stains should not be resolubilized in phosphate-buffered saline (PBS), but dilute solutions of the dye may be used with PBS or other phosphate-containing buffers. Solutions of Hoeschst dye should be stored at 2–6°C, protected from light. Stock solutions in water are stable for at least 6 months when refrigerated. For long-term storage the stock solution can be aliquoted and stored at ≤–20°C.

Caution

The Hoechst strains are known mutagens and should be handled with care. The dye must be disposed of safely and in accordance with applicable regulations.

Fluorescence Spectral Characteristics

The blue fluorescent Hoechst 33258, 33342, 34580 and nuclear yellow dyes may be efficiently excited with a xenon or mercury-arc lamp or with a UV laser; Hoechst 34580 may also be efficiently excited with non-UV wavelengths (>360 nm) such as the 405 nm laser line. They may all be detected using the common DAPI filter, blue GFP filters or the Semrock BrightLine® Alexa Fluor® 350 filter set.

Basic Protocol for Staining Cells

The following procedure can be adapted for most cell types. Note that different concentration ranges for the Hoechst dyes are suggested depending on the cell type (see Table 2). Growth medium, cell density, the presence of other cell types and other factors may influence staining. Residual detergent on glassware may also affect real or apparent staining of many organisms, causing brightly stained material to appear in solutions with or without cells present. Glassware should be washed in a mild detergent and rinsed with hot tap water followed by several rinses with deionized, distilled water.

Pellet cells by centrifugation and resuspend in buffered salt solutions or media, with optimal dye binding at pH 7.4. Adherent cells in culture may be stained *in situ* on coverslips. Add Hoechst stain using the concentrations listed in Table 2 as a guide. In initial experiments, it may be best to try several dye concentrations over the entire suggested range to determine the concentration that yields optimal staining. Unbound dye has its maximum fluorescence emission in the 510–540 nm range, this green fluorescence may be observed on samples using too high a concentration of dye.

Table 2. Recommended conditions for staining cells with Hoechst stains.

Cell Type	Hoechst Dye Concentration	Incubation Conditions
Bacteria	0.1 to 12 µg/mL	10 to 30 minutes
Live animal cells	0.2 to 5 µg/mL	20 to 30 minutes
Fixed animal cells	0.2 to 2 µg/mL	1 to 15 minutes

References

1. Nucleic Acids Res 15, 10589 (1987); **2.** Anal Biochem 147, 462 (1985); **3.** Anal Biochem 131, 538 (1983); **4.** J Histochem Cytochem 29, 326 (1981); **5.** Cytometry 3, 145 (1982); **6.** Cytometry 11, 386 (1990); **7.** Cancer Res. 48, 5742 (1988); **8.** Cytometry 11, 239 (1990); **9.** BBA 949, 158 (1988); **10.** Chromosoma 46, 255 (1974); **11.** J Histochem Cytochem 24, 24 (1976); **12.** J Histochem Cytochem 25, 913 (1977); **13.** J Mol Biol 315, 1049 (2002); **14.** Neurosci Lett 18, 19 (1980); **15.** Exp Brain Res 40, 383 (1980); **16.** J Histochem Cytochem 30, 123 (1982).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
F2962	FluoReporter® Blue Fluorometric dsDNA Quantitation Kit *200-2000 assays*	1 kit
N21485	nuclear yellow (Hoechst S769121, trihydrochloride, trihydrate)	10 mg
H1398	Hoechst 33258, pentahydrate (bis-benzimide)	100 mg
H3569	Hoechst 33258, pentahydrate (bis-benzimide) *10 mg/mL solution in water*	10 mL
H21491	Hoechst 33258, pentahydrate (bis-benzimide) *FluoroPure™ grade*	100 mg
H1399	Hoechst 33342, trihydrochloride, trihydrate	100 mg
H3570	Hoechst 33342, trihydrochloride, trihydrate *10 mg/mL solution in water*	10 mL
H21492	Hoechst 33342, trihydrochloride, trihydrate *FluoroPure™ grade*	100 mg
H21486	Hoechst 34580	5 mg
V13244	Vybrant® Apoptosis Assay Kit #5 *Hoechst 33342/propidium iodide* *200 assays*	1 kit
V23201	Vybrant® Apoptosis Assay Kit #7 *Hoechst 33342/YO-PRO®-1/propidium iodide* *200 assays*	1 kit

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